low (55 nM) and [$InsP_3$] was rapidly dropped from 10 μM to 0 using perfusion solution exchanges, the $InsP_3R$ channels had a high propensity to transiently enter a more active state before they became inactivated by the removal of $InsP_3$. This behavior was more prominent, with the channel P_o transiently increasing to ~ 0.6, when $[Ca^{2+}]_i$ was increased to optimal level (2.1 μM) instead of remaining constant at 55 nM. No transient increase in P_o was observed in similar [$InsP_3$] drops with $[Ca^{2+}]_i$ kept constant at 2.1 μM. This suggests that sub-saturating [$InsP_3$] can induce type 1 $InsP_3R$ channels to enter the high-P_o mode with higher probability than saturating [$InsP_3$].

Voltage-gated K Channels I

626-Pos Board B395

Discovery and Characterization of a Novel Toxin from Dendroaspis Angusticeps, Named TX7335, with an Activating Effect on the Potassium Channel KscA

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Due to their important role in essential physiological processes such as the propagation of the nerve signal or regulation of the heartbeat, potassium channels are common targets for animal toxins. These toxins provide valuable tools for the study of ion channel function and have potential as lead compounds for drug development. Most toxins affecting potassium channels act as pore blockers, thus inhibiting potassium flow. Using a direct pull-down toxin binding assay with immobilized channel (the bacterial potassium channel KcsA) and crude Dendroaspis angusticeps venom, we identified a novel toxin binder of KcsA, which we called Tx7335. Sequencing of the toxin by Edman degradation and mass spectrometry revealed a 63 amino acid residue peptide with 4 disulfide bonds that belongs to the family of the three-finger toxins, but with a unique modification of its disulfide-bridge scaffold. A functional analysis of Tx7335's effect on KcsA revealed a dose-dependent increase in both open probabilities and mean open times, thus leading to an increase in potassium conductance. Functional assays were also performed with a mutant of KcsA that mimicks the sequence of eukaryotic channels in the outer vestibule region and can bind the pore blocker charybdotoxin. This mutant showed the same susceptibility to the toxin, indicating that the binding site for Tx7335 is distinct from that of pore-blocker toxins. Given the fact that the toxin only showed an effect when added to the extracellular side of the channel, the binding site must be far removed from the intracellular pH gate. We therefore propose that Tx7335 increases potassium flow through KcsA by enhancing recovery from the inactivated state.

627-Pos Board B396

A KcsA-hERG Chimera Provides Structural Insights into the Unusual hERG Inactivation Gating Mechanism

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In order to provide some structural insights about the molecular basis of the inactivation gating of the hERG channel, we have reasoned that a chimeric approach in which we recreate the cavity of the hERG chanel in to the KcsA channel could potentially lends a structural framework to begin understand the hERG channel inactivation gating. Recently, we have made an important breakthrough by solving the X-ray structure of the bacterial K^+ channel KcsA trapped in the open and C-type inactivated state. These results allowed evaluating the structural changes underlying C-type inactivation gating in K^+ channels and it could lend a structural explanation to the unusual C-type inactivation process of the hERG channel. We have used the KcsA channel as a structural scaffold to introduce mutations at positions F103Y, G104A, L105S, V106I and L110V, making KcsA more hERG-like at positions (in hERG) Y652, A653, S654, I655 and V659. Additionally, we have solved the crystal structure of the closed (C) state for this KcsA-Herg chimera and surprisingly the selectivity filter in the closed state is collapsed. We have reasoned that the modifications introduced at the KcsA central cavity have strengthened the allosteric coupling between the activation gate and the selectivity filter, a similar phenomenon could underlies the unusual inactivation gating of the hERG channel. A comprehensive functional study will be presented in addition to a more complete structural analysis.

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628-Pos Board B397

Interaction of Local Anesthetics with KcsA K^+ Channel: Tetramer Stability and Docking Simulations

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Many local anesthetic and anti-arrhythmic drugs block voltage-dependent Na^+ and K^+ channels. In particular, cardiac Kv1.5 and Kv11.1 (hERG) exhibit drug- and K^+-dependent membrane stability. Since thermal stability of the KscA K^+ channel tetramer is K^+-dependent and all K^+ channels share a structurally conserved pore domain, we hypothesized that tetramer stability of KcsA may be sensitive to binding of local anesthetics. Using SDS-PAGE to monitor KcsA tetramer we found: 1. 20 mM Lidocaine (Lid) and 5 mM tetracaine (Tet) shift the temperature (T) dependence of tetramer stability to lower T in the presence of 5 mM K^+. 2. KcsA tetramer in the presence of 5 mM K^+ dissociates at 90° C as a function of [Lid] and [Tet]. 3. KscA tetramer completely dissociates in the presence of Tet (5 mM) but not Lid (20 mM) within 10 minutes at 22° C in the absence of K^+. 4. [K^+]-dependence of KcsA tetramer stability is shifted to higher [K^+] in the presence of Lid and Tet suggesting antagonism between binding of local anesthetics to a site in the tetramer and K^+ to the selectivity filter. These results are indicative of a negatively coupled interaction between local anesthetic drugs and K^+ that perturbs the tetrameric quaternary structure of K^+ channels. To investigate whether Lid and Tet could physically bind within the inner cavity of KcsA we carried out Monte Carlo-minimization docking of Lid and Tet to open, inactivated, and closed KcsA crystal structures. The results suggest that Lid and Tet binding within the inner cavity of KcsA involves electrostatic repulsion with K^+ and non-bonded attraction to residues of the selectivity filter (Thr74, Thr75) and inner pore helix (Ile100, Phe103).

629-Pos Board B398

Mechanisms of Fast Blockade and Gating of the MthK Channel Pore by Divalent Cations

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K^+ channels can undergo inactivation, a process in which K^+ conduction decays over time, contributing to tuning of electrical excitabilty in nerve and muscle. Although K^+ inactivation is associated with conformational changes in the channel pore, these are likely to be preceded by interactions between the pore and surrounding ions. Here we use a series of divalent metal cations to systematically probe these interactions in MthK, a K^+ channel of known structure, by electrophysiological methods and molecular dynamics simulations. Mg^{2+}, Ca^{2+}, and Sr^{2+} each yielded roughly equivalent levels of fast blockade, with similar voltage dependences (delta ~0.2). In contrast, Ca^{2+} and Sr^{2+} were found to substantially enhanced voltage-dependent inactivation, whereas Mg^{2+} did not. The inactivation enhanced by Ca^{2+} or Sr^{2+} was not consistent with a simple voltage-dependent slow blockade, because increasing either [Ca^{2+}] or [Sr^{2+}] to 20 mM yielded little additional inhibition compared with the level produced at 5 mM, over the same voltage range. The differential effects of Ca^{2+}/Sr^{2+} vs. Mg^{2+} on inactivation suggests that the slightly larger divalent cations enhance inactivation through a site that is separate from the one responsible for fast blockade. Molecular dynamics simulations and potential of mean force calculations suggest that Mg^{2+}, Ca^{2+}, and Sr^{2+} each can access a site in the wide cavity of the MthK pore (S_{cav}) whereas Ca^{2+} or Sr^{2+} (but not Mg^{2+}) are likely to further access a site at the entry to the selectivity filter (S5). We hypothesize that a divalent cation at S5 can affect a redistribution of K^+ ions in the selectivity filter, which may in turn inhibit the conduction cycle and enhance inactivation.

630-Pos Board B399

A Novel Interaction in the Selectivity Filter of Voltage-Gated Potassium Channels Crucial for Slow Inactivation

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